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RESEARCH ARTICLE

The serpin PN1 is a feedback regulator of FGF signaling in germ layer and primary axis formation

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ABSTRACT

Germ layer formation and primary axis development rely on Fibroblast growth factors (FGFs). In *Xenopus*, the secreted serine protease HtrA1 induces mesoderm and posterior trunk/tail structures by facilitating the spread of FGF signals. Here, we show that the serpin Protease nexin-1 (PN1) is transcriptionally activated by FGF signals, suppresses mesoderm and promotes head development in mRNA-injected embryos. An antisense morpholino oligonucleotide against PN1 has the opposite effect and inhibits ectodermal fate. However, ectoderm and anterior head structures can be restored in PN1-depleted embryos when HtrA1 and FGF receptor activities are diminished, indicating that FGF signals negatively regulate their formation. We show that PN1 binds to and inhibits HtrA1, prevents degradation of the proteoglycan Syndecan 4 and restricts paracrine FGF/Erk signaling. Our data suggest that PN1 is a negative-feedback regulator of FGF signaling and has important roles in ectoderm and head development.

KEY WORDS: SerpinE2, HtrA1, Syndecan, FGF, Early development, *Xenopus*

INTRODUCTION

The question of how the body plan acquires proper proportioning of its germ layers and subdivides the primary axis into head, trunk and tail structures is of fundamental importance in developmental biology. In *Xenopus*, mesoderm formation occurs after the midblastula transition, when signals from the vegetal endoderm, primarily members of the Nodal-related class of the TGFβ family and Fibroblast growth factors (FGFs), induce mesoderm from competent ectoderm in the adjacent marginal zone (De Robertis et al., 2000; Kimelman, 2006). At the advanced gastrula stage, posterior mesoderm secretes signals such as Wnts and FGFs, but also Nodals and BMPs, that convert head into trunk and tail structures (Niehrs, 2004; Pera et al., 2014). The ectoderm and head are considered as default fates that form in the absence of growth factor signaling.

FGFs stimulate mesoderm induction and posterior development via the Extracellular signal-regulated kinase (Erk) pathway (Amaya et al., 1991; Umbhauer et al., 1995). Fgf4 and the T-box transcription factor *Xenopus* brachyury (Xbra) engage in a positive feedforward loop that causes amplification of the mesoderm-inducing and posteriorizing signal (Isaacs et al., 1994; Schulte-Merker and Smith, 1995). Although negative-feedback mechanisms for FGF signals exist in the mesoderm (Böttcher and Niehrs, 2005), it is not clear how the ectoderm protects itself against self-propagating FGF signals. Whether FGF signals need to be suppressed to allow head formation has not convincingly been demonstrated.

Proteoglycans through their heparan sulfate (HS) chains capture FGFs at the cell surface, regulate their extracellular transport to target cells and participate in complex formation with their receptors (Yu et al., 2009; Matsuo and Kimura-Yoshida, 2013). We previously described an autoinductive loop of FGF and the secreted serine protease HtrA1 that leads to the mobilization of FGF/proteoglycan complexes and long-range FGF signaling during mesoderm induction and posteriorization in *Xenopus* embryos (Hou et al., 2007). FGFs stimulate *HtrA1* transcription also in the chick (Ferrer-Vaquer et al., 2008) and zebrafish (Kim et al., 2012). It is apparent that the proteolytic activity of HtrA1 needs to be regulated to protect the integrity of proteoglycans and ensure proper FGF signaling in the embryo.

In a direct screen for secreted proteins in early *Xenopus* embryos, we identified Protease nexin-1 (PN1) (Pera et al., 2005). PN1, which is also known as Glial-derived nexin or SerpinE2, is a serine protease inhibitor (serpin) that contains an exposed reactive center loop (RCL) that covalently binds to and blocks proteases such as thrombin, plasminogen activator, trypsin, urokinase and factor XIa (Baker et al., 1980; Stone et al., 1987; Knauer et al., 2000). Serpins inhibit target proteases through a suicide substrate mechanism, by which the protease cleaves the RCL at the process site and forms a covalent acyl-enzyme complex that causes irreversible inhibition of the protease (Olson and Gettins, 2011; Li and Huntington, 2012). *PN1* null mice exhibit increased proliferation in the postnatal cerebellum (Vaillant et al., 2007), but no apparent early developmental defects have been described. In *Xenopus* embryos, overexpression of PN1 inhibits convergence extension movements and the expression of mesendodermal markers (Onuma et al., 2006). Here, we show that *PN1* gene activity is positively regulated by FGF signals and is crucial for the formation of ectoderm and head structures. We report that PN1 binds and inhibits HtrA1, regulates the turnover of Syndecan 4 (Sdc4) and controls the range of FGF/Erk signaling. Thus, our study uncovers a novel important role of PN1 as negative-feedback regulator in the extracellular regulation of the HtrA1-FGF axis during germ layer and primary axis development in *Xenopus*.

RESULTS

Two *Xenopus PN1* genes are partially co-expressed with *HtrA1* and activated by FGF signals

In a screen for secreted proteins from gastrula stage *Xenopus laevis* embryos, we previously isolated five non-redundant full-length

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